

AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph beginning on page 1, line 5, as follows:

This application is a divisional of U.S. Serial No. 09/893,191, filed June 26, 2001, now **allowed** issued as U.S. Patent No. 6,686,156, which claims the priority benefit of the provisional patent applications U.S. Serial No. 60/213,213,908, filed June 26, 2000, and U.S. Serial No. 60/277,748, filed March 21, 2001, both of which are incorporated herein by reference in their entirety.

Please amend the paragraph beginning on page 88, line 14, as follows:

Spacer18-

Spacer18CGGTACGCTGATCAAAGATCCGTGCAACAAATGTCA

TGGTCATGGTCGTGTTGAGCGCAGCAAAACGCTGTCCGT TAAAATCCCGGCAGGGGTG
GAACTGGAGACCGCATCCGT (SEQ ID NO:3). Spacer18 refers to polyoxyethylene spacers. These were added to the oligo in order to retard its mobility with respect to the 100-bp ssDNA product. The sequence of the aforementioned primarily single-stranded DNA (351 bases) template produced by PCR amplification is:

CGGTACGCTGATCAAAGATCCGTGCAACAAATGTCATGGTCATGGTCGTGTTGAGCG
CAGCAAAACGCTGTCCGT TAAAATCCCGGCAGGGGTGG**AACTGGAGACCGCATCCGT**
CTTGCGGGCGAAGGTGAAGCGGGCGAGCATGGCGCACCGGCAGGCGATCTGTACGTTC
AGGTTCAAGTTAAACAGCACCCGATTTTCGAGCGTGAAGGCAACAACCTGTATTGCGA
AGTCCCGATCAACTTCGCTATGGCGGCGCTGGGTGGCGAAATCGAAGTACCGACCCTT
GATGGTCGCGTCAAACCTGAAAGTGCCTGGCGAAACCC**AGACCGGTAAGCTATTCCGT**
ATGCG (SEQ ID NO:4) wherein the PCR primers are bolded and underlined and the composite primers are bolded, with RNA portion in italics.

Please amend the paragraph beginning on page 89, line 3, as follows:

Single-stranded DNA template for amplification was prepared by PCR amplification of a 351-bp segment of the *E. coli* J gene using the primers IA006 and IA004. Primer IA006 is a 23-mer with the sequence of: CGGTACGCTGATCAAAGATCCGT (SEQ ID NO:5). Primer IA004 is a 26-mer with the sequence of: CGCATACGGAATAGCTTACCGGTCT (SEQ ID NO:6).

Please amend the paragraph beginning on page 91, line 3, as follows:

Oligonucleotide probes for hybridization examples(IA010 for ssDNA products; IA014 for ssRNA products) were 5'-end-labelled using T4 polynucleotide kinase (New England Biolabs, Beverly, MA) and γ -³²P-ATP (adenosine 5'-[γ -³²P] triphosphate, triethylammonium salt, Amersham, Piscataway, NJ; PB10218, >5000 Ci/mmol, 10 mCi/ml). Primer IA010 is a 21-mer with the sequence of: ATGTCATGGTCATGGTCGTGT (SEQ ID NO:7). Primer IA014 is a 31-mer with the sequence of: CTCAACACGACCATGACCATGACATTTGTTG (SEQ ID NO:8). Labelling reactions (50 μ l total volume) contained 70 mM Tris-HCl, pH 7.6, 10 mM MgCl₂, 5 mM DTT, 1 μ g oligo (147 pmol for primer IA010; 101 pmol for primer IA014), 250 μ Ci γ -³²P-ATP, and 30 Units T4 polynucleotide kinase. Incubation was at 37°C for 30 minutes, followed by removal of unincorporated nucleotide using QIAquick Nucleotide Removal Kit (Qiagen, Valencia, CA). The decay rate (cpm) was determined in a Packard Minaxi Tri-Carb 4000 Series liquid scintillation counter by Cherenkov counting of 1 μ l of the labelled oligo.

Please amend the paragraph beginning on page 92, line 15, as follows:

Probe IA010 hybridization and autoradiography to the ssDNA product of the linear amplification method, verified the identity of the amplification product. The linear amplification of the synthetic oligonucleotide target in this experiment was done using a non blocked promoter-template oligonucleotide (IA015b). Promoter-template oligonucleotide IA015b is a 55-mer with a sequence of:

GGAATTCTAATACGACTCACTATAGGGAGAGCGGTACGCTGATCAAAGATCCGTG (SEQ ID NO:9). The standard reaction components used for this amplification reaction are as given

above. The initial denaturation step was performed at 70°C for 10 seconds. The reactions were cooled down to 65°C, and further incubated at this temperature for 30 minutes following the addition of Bca polymerase and RNase H. No hybridization was detected in the control reactions (no DNA, no primer, no RNase H, no Bca).

Please amend the paragraph beginning on page 92, line 29, as follows:

The promoter-template oligonucleotide (PTO) contains two essential sequence motifs: a T7 promoter sequence (5'-TAATACGACTCACTATAGGGAgGAG) (SEQ ID NO:10) and a sequence complementary to the ssDNA template. Four versions of a PTO were designed (IA012, IA012b, IA015, IA015b). IA012 PTO is a 67-mer and has a sequence of:

GGAATTCTAATACGACTCACTATAGGGAGAGATCGAGTAGCTCCGGTACGCTGATCAA
AGATCCGTG (SEQ ID NO:11)[[.]] . IA012 PTO contains two sequences in addition to the core

T7 promoter: a 5'-extension (5'-GGAATTC) and a spacer (5'-ATCGAGTAGCTC) between the promoter and the target DNA-complementary sequence. IA015 is the shorter PTO (48-mer), lacking both the 5'-extension and the spacer. IA015 PTO has the sequence of:

TAATACGACTCACTATAGGGAGAGCGGTACGCTGATCAAAGATCCGTG (SEQ ID
NO:12). IA012b PTO is a 60-mer which contains the spacer, but not the extension. IA012b PTO has the sequence:

TAATACGACTCACTATAGGGAGAGATCGAGTAGCTCCGGTACGCTGATCAAAGATCCG
TG (SEQ ID NO:13). IA015b contains the extension, but not the spacer. The sequence of IA015b is disclosed in Example 2. All primers other than the chimeric oligonucleotides IA005, IA019, and IA020 were synthesized by Keystone (Division of BioSource International, Camarillo, CA) and were PAGE purified.

Please amend the paragraph beginning on page 93, line 18, as follows:

The ability of IA012, IA012b, IA015, and IA015b to convert the ssDNA template into a substrate for T7 RNA polymerase was assessed by comparing the amount of RNA produced after transcription of overlap-extension products formed between a synthetic oligo product (IA009) and

each of the PTO's. Synthetic oligo product IA009 is a 100-mer with the sequence of:

AGTGTCCACCCCTGCCGGGATTTTAACGGACAGCGTTTTGCTGCGCTCAACACGACCAT
GACCATGACATTTGTTGCACGGATCTTTGATCAGCGTACCG (SEQ ID NO:14). Overlap-extension was performed in 15 ul reactions containing 20 mM Tris-HCl, pH 8.5, 6 mM MgCl₂, 1 mM each dNTP (dATP, dTTP, dCTP, dGTP), 100 nM IA009, 100 nM PTO, and 1 Unit Bca DNA polymerase. Reactions were constituted without Bca DNA polymerase, heated to 95°C then cooled over 10 minutes to 60°C. After addition of DNA polymerase, reactions were incubated at 60°C for 30 minutes. A portion (2.5 µl) of the reaction mixture was added to the standard RNA transcription reaction mixture and the transcription reactions were assessed by gel electrophoresis.

Please amend the paragraph beginning on page 97, line 3, as follows:

The performance of each of the three composite primers in the first transcription module of composite primer-based amplification methods of the invention was assessed. The isothermal linear amplification was performed in 15 µl reactions containing 20 mM Tris-HCl, pH 8.5, 6.0 mM MgCl₂, 1.0 mM dATP, 1.0 mM dCTP, 1.0 mM dTTP, 0.8 mM dGTP, 0.2 mM dITP (dNTP's from Amersham), 6% DMSO, 8% glycerol, 100 µg/ml acetylated BSA (Ambion, Austin, TX), 0.6 Units/µl recombinant ribonuclease inhibitor (rRNasin, Promega, Madison, WI), 5 µM composite primer, 200 nM promoter-template oligonucleotide (PTO) IA015C. The sequence of PTO IA015C is disclosed in Example 1. The sequence of composite primers IA005 (20-mer) is disclosed in Example 1. Other composite primer sequences with alphanumerical names are as follows:

IA019 (20-mer) ACGGAUGCGGUCUCCAGTGT (SEQ ID NO:15)

IA020 (21-mer) GACGGAUGCGGUCUCCAGTGT (SEQ ID NO:16)

Four other composite primer ~~sequence~~ sequences were used that did not have alphanumerical names. Their sequences are, respectively:

(1) GCAAGACGGAUGCGGUCUCCAGTGT (SEQ ID NO:17)

(2) GACGATGCGUCTCCAGTGT (SEQ ID NO:18)

(3) GACGGATGCGGUUCTCCAGUGT (SEQ ID NO:19)

(4) GACGGATGCGGUUCTCCAGUGUCCA (SEQ ID NO:20)

Please amend the paragraph beginning on page 104, line 16, as follows:

The sequences used in this example are as follows:

composite RNA/DNA primers

IA005	r{ACggAUGCggUCUCC} AgTgT (<u>SEQ ID NO:15</u>)	(20-mer)
IA019	r{ACggAUGCggUCUCCAg} TgT (<u>SEQ ID NO:15</u>)	(20-mer)
IA020	r{gACggAUGCggUCU} CCAgTgT (<u>SEQ ID NO:16</u>)	(21-mer)

Please amend the paragraph beginning on page 105, line 4, as follows:

IA015c (55-mer):

ggAATTCTAATACgACTCACTATAgggAgAgCggTACgCTgATCAAAGATCCgTg-biotin (SEQ ID NO: 2)

Please amend the paragraph beginning on page 105, line 8, as follows:

CggTACgCTgATCAAAGATCCgTgCAACAAATgTCATggTCATggTCgTgTTgAgC
gCagCAAAACgCTgTCCgTTAAAATCCCggCaggggTggACACTggAgACCgCATCCgTCTTgCg
ggCgAAggTgAAgCgggCgAgCATggCgCACCggCaggCgATCTgTACgTTCaggTTCaggTTAAA
CAgCACCCgATTTTCgAgCgTgAAggCAACAACCTgTATTgCgAAgTCCCgATCAACTTCgCT
ATggCggCgCTgggTggCgAAATCgAAgTACCgACCCTTgATggTCgCgTCAAAGTgAAAATgCC
TggCgAAACCCAgACCggTAAgCTATTCCgTATgCg (SEQ ID NO:4)

Please amend the paragraph beginning on page 105, line 18, as follows:

IA009 (100-mer):

AgTgTCCACCCCTgCCgggATTTTAACggACAACgTTTTgCTgCgCTCAACACgACCATgACCA
TgACATTTgTTgCACggATCTTTgATCAgCgTACCg (SEQ ID NO:14)